MISSION-T2D

Multiscale Immune System SImulator for the Onset of Type 2 Diabetes integrating genetic, metabolic and nutritional data

Work Package 2

Deliverable 2.5

Report on the search of inflammatory cytokines capable to impact the balance of metabolic flexibility

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Executive Summary	In this document are reported the results of the Task 2.1 with specific emphasis on the search of inflammatory cytokines capable to impact the balance of metabolic flexibility. The work reported here regards the activities of identification of the main inflammatory mediators and regulators that interact with metabolic flexibility and homeostasis. The main goal is to provide a detailed framework of the most relevant molecular players and cell/organs interactions that can help in interpreting and classifying the results of the simulations.
Keywords	Metaflammation, inflammation, mediators, cytokines, metabolic flexibility

Contents

1. Deliverable Description

In this document the results of the search of inflammatory cytokines capable to impact the balance of metabolic flexibility are described. In the present report we will summarize the results coming from an in depth analysis of the literature regarding the main inflammatory mediators and regulators involved in metabolic flexibility and homeostasis especially in the condition of high nutrient intake. This activity started from the very beginning of the project and its main goal was to provide, in the first phases, the immunological background to the modelling team for the set up of the agent-based model of the immune system in response to high nutrient intake, and later on, for the interpretation and classification of the simulation results. This activity has required an in-depth analysis of the available literature and numerous meeting with the modelling team to integrate this knowledge in the model and to extract insights from the simulations, relevant to metabolic flexibility.

2. Deliverable Results

At the basis of the theoretical background that sustains the whole MT2D project there is the critical role that inflammation and immune system play in the etiology of sporadic type 2 diabetes. While it is well accepted since many years that inflammation is a key player in T2D, only recently convincing theories and experimental data emerged, shedding light on possible mechanisms that link the immune system with the metabolism flexibility and eventually with metabolic derangement and diabetes.

The most advanced and convincing mechanisms can be recapitulated in the so-called metaflammation process (M. Gregor, 2011), that is a low-grade chronic inflammatory status, that is likely sustained by high nutrient intake that alter the inflammatory milieu of metabolic cells and tissues. The two major players here are the adipose tissue and the liver and a key role is played by stress sensors like JNK and IKK.

On the whole the metaflammation will exacerbate the insulin secretion by promoting local insulin resistance in several tissue and organs. Accordingly metaflammation increase the stress on the pancreatic βcells consistently contributing to their damage and eventually depletion.

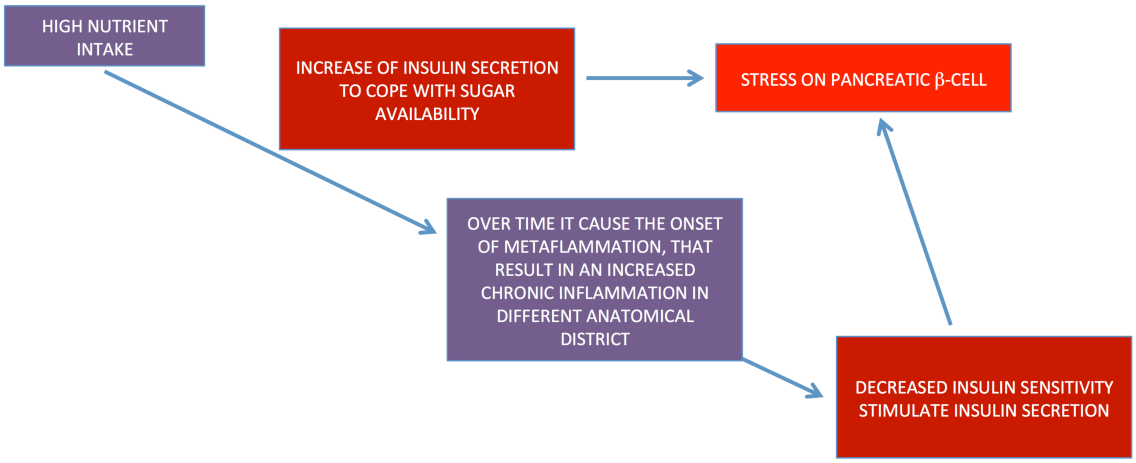

Fig. 1: the figure represents a schematic diagram of the general mechanisms that link diet and inflammation toward T2D onset.

Before going in depth in the analysis of metaflammation framework, it is worth mentioning that there are still huge gaps in the comprehension of metabolic instability and of the T2D etiology. Indeed, while the metaflammation theory and the other molecular interactions between nutrition/nutrients and inflammation are sound and convincing, there are some natural phenotypes that show the limits of such mechanistic hypothesis, such as the healthy extreme obesity phenotype that completely unfits the metaflammation frame and underlines the fact that the interactions between the individual genetic background and environment are determinant for the onset of diabetes, and are far from being elucidated.

Another critical issue that also potentially hampers the in-silico studies is that there is little knowledge regarding the timeframe of metaflammation set up. Since the metaflammation phenotype is dependent by chronic, long-term processes, it is very difficult to assess the nutrient excess load and the exposure time necessary to activate the metabolic-driven inflammation.

3. Metaflammation

The term metaflammation indicates a condition of low-grade inflammation that is driven by metabolic dysfunction processes. Metaflammation generally inhibits insulin signalling in metabolic tissues. The excess in nutrients can indeed activate cytokines and/or TLR receptors, that in turn transduce the signal to the kinases JNK, IKK and PKR that inhibit insulin signalling through phosphorylation of insulin receptor substrate 1 IRS-1.

There are several evidences that indicate that the daily nutrient intake somehow stimulates low-grade inflammatory response. The causes and the mechanisms behind

this stimulation are still not clear, two major hypothesis are present:

i) since the nutrients come from the environment, they stimulate a low level inflammatory response since are not recognized as self. (Wellen, 2007; Gregor, 2011)

ii) from an evolutive point of view, the immune system is naturally stimulated by nutrient intake and takes an active and physiological part in nutrition and metabolism.

An example of the direct role of nutrients in fuelling inflammatory response is the modulation of NLPR3 inflammasome by dietary fatty acids. In human monocytes palmitic acid promotes the heterodimerization of TLR1 and TLR2, inducing NF-kB mediated expression of pro-IL1b and NLRP3 and activation of the inflammasome, which in turns promotes the activation and the release of IL1b (Snodgrass, 2013). On the contrary, stimulation of macrophages with the omega-3 fatty acid DHA inhibits TLR1-TLR2 heterodimerization and abolishes NLRP3 inflammasome activation (Yan Y., 2013).

The general hypothesis is that while the regular nutrient consumption does not alter the physiological state, the chronic nutrients excess and in particular the consumption of a high fat diet leads to an increase of the inflammatory response, that over time in turn results in the activation of the immune system cells in metabolic tissues such adipose tissue and in liver. The activation of the immune system effectors in the metabolic tissue contributes to generate different local inflammatory foci that overall increase the pro-inflammatory tones where the insulin action is more critical.

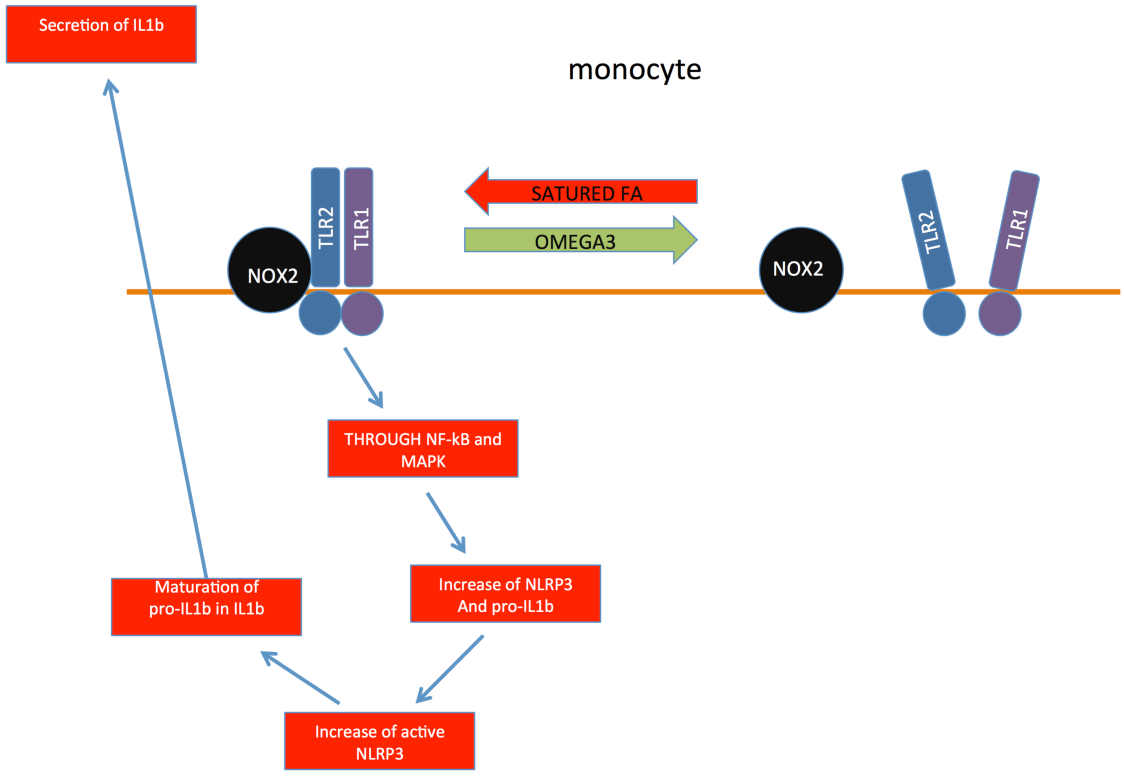

Fig. 2: in the figure a schematic representation of the interaction between saturated and unsaturated fatty acids on NLRP3 inflammatory pathway is shown. In particular is shown that saturated fatty acids are recognized by TLR1 and 2, that in turn promote NLRP3 expression, that leads to the IL1b secretion.

The emerging view is that specialized metabolic cells (adipocytes, hepatocytes, myocytes *in primis*) are capable of engaging inflammatory response to nutrient excess (Gregor M, 2011). This perspective shows clearly that to provide a step further in the comprehension of the interactions between the immune system and metabolism, in the presence of chronic nutrient excess, the "local" dimension has to be carefully considered. This is the reason that leaded to the implementation of organ agents in the simulator, able to provide inflammatory feedback in response to chronic overfeeding.

4. Adipose Tissue

The adipose tissue is at the heart of metaflammation. The nutrient-related inflammatory alterations were first described in the adipose tissue. The excess of nutrients can induce functional stress in the cell through several mechanisms, leading to inflammatory responses.

One intriguing scenario is that the increase in volume of lipid droplets within the adipocyte leads the cell to its mechanical limit for storage capacity. Under these conditions the adipose tissue is less vascularized and the stressed adipocytes undergo cellular death, releasing cytokines and excess fatty acids, that in turn fuel the

inflammatory cascade (Gregor, 2011).

Another key component in this process is organelle stress, and in particular endoplamic reticulum (ER) stress. It has been shown that adipose tissue shows increased levels of ER stress (Ozcan, 2004), which leads to the activation of the unfolded protein response (UPR). Notably, three main ER transmembrane enzymes responsible for UPR (PERK, IRE-1 and ATF-6) can activate several inflammation-related molecules, including JNK and IKK, leading to increased expression of inflammatory cytokines (Hu, 2006; Urano, 2000), NF-kB and PKR. The activation of NF-kb sustains the increase in expression of IL-1b mRNA. In addition, it has been recently demonstrated that ER-stress can activate NLTP3 inflammasome, resulting in IL-1b activation through its cleavage and secretion (Kim, 2013).

Different studies clearly showed that the increase of pro-inflammatory mediators, such as TNF alpha, in adipose tissue leads to insulin resistance in this anatomical district (Hotamisligil, 1994; Engelman, 2000; Stephens, 1997). In addition it was shown that the effect of excess of proinflammatory molecules interferes not only with the activity of insulin receptor in adipocytes but, in addition, it can also downregulate the activity of the nuclear receptor peroxisome proliferator-activated receptor γ (PPARγ), which is essential to adipogenesis and to maintenance of adipocyte gene expression and function (Guilherme, 2008). This compromises the cell ability to store lipids and reduces adiponectin expression, affecting insulin sensitivity.

The pro-inflammatory milieu promotes the infiltration of immune cells, primary macrophages. Several studies in animal models sustain a model in which the immune system has an important role in disrupting the metabolic function and in promoting insulin resistance (Odegaard, 2007; Kang, 2008). Accordingly, the inactivation or the removal of immune cells result beneficial for anabolic insulin pathways (Liu, 2009). More recently, it has been shown that production of IL-12 and IL-18 in the adipose tissue promotes the accumulation and the activation of T lymphocytes, especially CD8+ cells (Nam, 2013; Jiang, 2013).

Finally, it is worth mentioning that adipocytes express TLR receptors, in particular TLR4 and TLR2, that can be activated by the binding of saturated fatty acids triggering, as described above, a signal transduction cascade which results in insulin resistance (Gregor, 2011).

5. Liver

As in adipose tissue, also in the liver, the other major metabolic organ, inflammatory

mediators are involved in insulin resistance. In this case however, metaflammation is sustained by the activation of the macrophage-like Kupffer cells resident in the liver, and not by the infiltration of immune cells.

Like in the adipose tissue, inflammation-induced insulin resistance is triggered by the activation of NF-kB (Cai, 2005), affecting gluconeogenesis and contributing to hyperglycaemia. Also in this tissue, the repression of the inflammatory response restores insulin sensitivity and gluconeogenesis. The increased levels of pro-inflammatory cytokines could be at basis of hepatosteatosis that is an harmful phenotype that is often associated with obesity as shown by a couple of studies showing that the administration of IL6 and TNF-α induced liponeogenesis and triglyceride production in liver. It is noteworthy that in obese liver is increased the secretion of inflammatory mediators and acute phase reactant such as CRP, PAI-1 and IL6, compared to lean controls (Pickup, 1998; Shoelson, 2006).

6. Pancreas

Peripheral insulin resistance promoted by nutrient excess strongly challenges β cells function, pushing their proliferation and resulting in cellular stress and finally in apoptosis. This condition sustains the expression of inflammatory cytokines in the pancreas, promoting macrophage infiltration and glucose intolerance (Ehses, 2002).

It has been recently demonstrated that activation of NLRP3 inflammasome in macrophages by endocannabinoids sustains the production of inflammatory cytokines, including IL-1β (Jourdan, 2013). In particular, IL-1β and IFN-γ activate the NF-κB pathway and JNK signaling, which affect insulin production and survival of β cells.

7. Brain

Brain plays a major role in the metaflammation circuitry. Even though brain cannot be considered a metabolic organ, it regulates feeding behaviour, thus it is responsible for the chronic excess in nutrient intake that is at the basis of the disruption of the physiological relationship between metabolism and immune system.

In particular the hypothalamus is responsible to for nutrient sensing and the regulation of satiety feeling in response to insulin from pancreas and to leptin, which is secreted from adipocytes.

The first strong relationship with metaflammation is the insulin and leptin resistance that is often observed in obese patient. It is observed in different studies that in obese persons there is a reduced effect of insulin and leptin in reducing the hunger stimulus.

This impaired mechanism generates an imbalance between the insulin and leptin secretion, that is proportional to nutrient intake, and the satiety, sustaining detrimental feeding behaviour over time and allowing the onset of metaflammation (Obici, 2002; Obici, 2003).

There are evidences that this resistance could be related to hypothalamus impairment due to local brain inflammation again activated by nutritional triggers. Comparing the hypothalamus of HFD with lean animals it emerged that HFD present high levels of pro-inflammatory compounds such as TNF-α, IL-1β, and IL-6 accompanied by increased apoptotic markers (Moraes, 2009). Despite this is one of the most intriguing topic in the field of metabolic diseases, few studies are available. Some evidences in mice showed that to activate this peculiar kind of neuro-inflammation the TLR pathway is mainly involved.

Again, the mechanism is the recognition by TLR molecules of satured fatty acid as exogenous molecules, leading to the activation of downstream inflammatory responses including NLRP3 activation. Accordingly, experiments showed that the local inhibition of TLR molecules in the brain restored leptin signalling and reduced overfeeding behaviour in HFD mice (Milanski, 2009; Kleinridders, 2009; Sabio, 2010; Belgardt, 2010).

8. Gut

One of the most recent topics in the field of metabolic diseases is the role of gut and in particular of gut microbiota. In this new perspective the gut microbiota has to be considered as part of the system and in such perspective the individuals have to be considered as meta-organisms.

The host-bacteria interaction in the contest of HFD and obesity are many, including a consistent effect on the overall inflammatory tone. In particular several studies showed that in obese human and animals a decrease of *Bacteroidetes* and a concomitant increase of *Firmicutes* is observed. These ecological changes are reversible by diet interventions (Ley, 2006).

The potential role of gut microbiota is still a matter of debate, but a remarkable study showed that the obese mice microbiota transplanted in lean mice is sufficient to increase body weight, suggesting that this shift is responsible for metabolic imbalance possibly also by altering the inflammatory milieu (Turnbaugh, 2006). This result was somehow confirmed by a study that showed how germ-free mice show metabolic derangement, including insulin resistance, under HFD conditions (Backhed, 2007). It

was demonstrate that the obese gut microbiota stimulates the increase of IL1-β and TNF-α and with age also an increase of IL8 is observed (Vijay-Kumar, 2010).

9. Muscle

Muscles are among the tissues that present the highest glucose uptake. This uptake is obviously related to physical activity that is frequently very limited in obese patients. To date no evidences existing regarding a role of obesity in inducing inflammation in muscle.

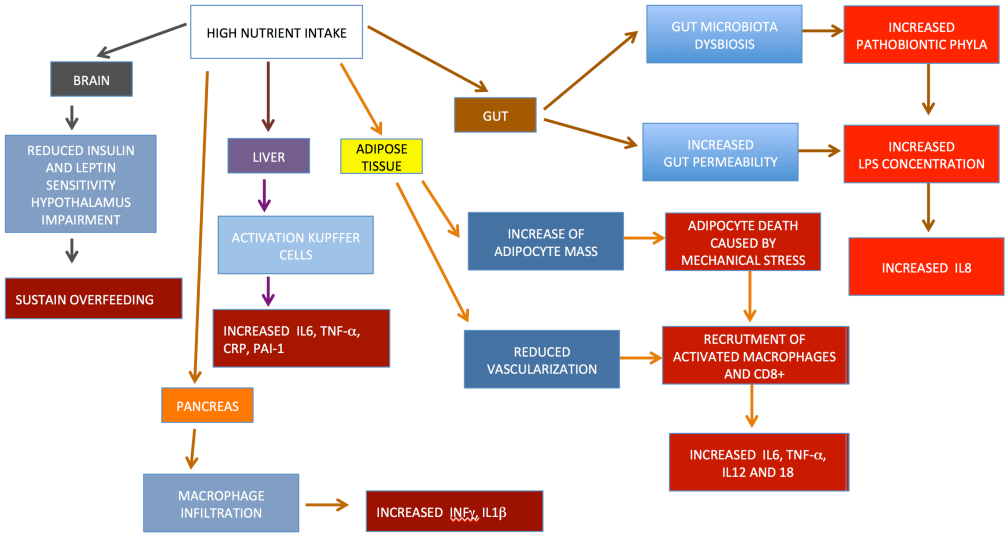

Fig. 3: in figure we find a schematic representation of the interaction that high calorie/fat diet has on inflammation. The scheme take into account all the different anatomical districts that presents a direct and specific diet/inflammation interaction. The basic molecular mechanism and the specific inflammatory path are described.

TISSUE	EFFECT OF NUTRITION EXCESS	INFLAMMATION
ADIPOSE TISSUE	• Increased adipocyte mass • Reduced vascularization • Adipocyte death • Macrophage and CD8+ infiltration	Increased iINFγ–, TNF-α, IL-6, IL-12 and IL-18
GUT	• Dysbiosis toward increase of pathobiontic species • Increased gut permebility	Increased IL-8, TNF-α, LPS, IL-1β
MONOCYTE	• Increase of nlrp3 activation through satured fatty acids stimulation by tlr2 and tlr3	Activation of TLR receptor in response of satured FA, Increase of IL-1β

LIVER	• Activation of Kupfer cells	Activation of NF-kB Increased IL-6, TNF-α, CRP, PAI-1
BRAIN	• Resistance to insulin and leptin satiety stimulation • Impairment of hypothalamus	Activation of TLR receptor in response of satured FA, Increase of IL-1β
PANCREAS	• Increased inflammatory mediators • Macrophage infiltration	Increased INF-γ, IL-1β

Table 1: the table summarize the basic information of the interaction between high calorie/fat diet and inflammation. The information are subdivided according the different anatomical districts that presents a direct and specific diet/inflammation interaction. The basic molecular mechanism and the specific inflammatory path are described.

10. Deliverable Conclusions

In the present report we summarized the results of the in-depth analysis of the literature regarding the role of inflammation in hampering metabolic homeostasis under nutrient excess condition. With this analysis we provided a broad overview of the behaviour of the most important inflammatory compounds in the condition of obesity and nutrient excess, linking such behaviour to the metabolic flexibility derangement that occur in such frame, that is prodromal to the onset of type T2D.

Framing the above described inflammatory and metaflammatory response in the context of different tissues and conditions (namely, nutrient excess, unbalanced dietary intakes, physical activity) is critical for the interpretation of the simulation results, whose concomitant in-depth analysis will be object of a focused study based on the project results (article in preparation).

Indeed, the time-dependent dynamics of several variables here described are tracked with respect to changes of initial conditions and key input parameters (i.e., user input, namely, initial physical state, physical activity and dietary habits, see MT2D Deliverable D6.3, Fig. 1). We very briefly account here about two conditions that are critical to the understanding of the T2D onset.

The first, according to the biological framework described here, is referred to regular energy consumption, which does not impact sufficiently on the metabolic state when in presence of chronic nutrients excess: simulations show that the physical activity alone does not affect significantly inflammation (inflammatory markers) arising from a poor quality nutritional style (MT2D Deliverable D6.3).

The second issue, which has been mentioned above and it is also able to potentially

hampers in-silico studies, is that there is little knowledge regarding the time frame of metaflammation set up. Since the metaflammation phenotype is dependent by chronic, long-term processes, it is very difficult to assess the nutrient excess load and the exposure time necessary to activate the metabolic-driven inflammation. In this case, it has been demonstrated (Kardinaal, 2015) that short-term (4 weeks) hyper-caloric intervention, while inducing anthropometric and metabolic changes in adipose tissue mass and function, metabolic flexibility, and glucose metabolism in healthy subjects in fasting state, does not significantly alter major biomarkers of metabolic health (e.g., glucose, TGs, and CRP). Simulation data agree on this picture, since equivalent variables' values were practically unchanged after OLTT, but showed a slight increase during the HFHC diet (MT2D Deliverable D6.3, Fig. 13). This is coherent with the picture that healthy subjects develop inflammatory stress only over a longer period, condition that cannot be evidenced in shorter, 4 weeks interval.

From the analysis it appeared clear that the metaflammation process is extremely complex. This complexity is mainly related to the fact that the events that are at the aetiological basis of such phenotype are dispersed in different tissues and organs and that only long-term chronic exposure to nutrient excess is able to establish metaflammation.

Accordingly, it is worth to remember that, while the overall picture of metaflammation is rather convincing and attractive, the evidence that sustain the different pillars of the theoretical frame are often scarce and not replicated.

One of the most far reaching conclusion that we could derive from our analysis could be that on the whole the metabolically driven chronic inflammation is critical for the onset of T2D, and since it is locally determined by peculiar cellular and molecular mechanisms an effective therapeutic approach to extinguish this destructive flame should consider actions that concomitantly are effective in counteracting all the different foci of inflammation, taking into account that all the different foci present peculiar inertia and latency in responding to anti-inflammatory therapies.

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